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权利要求书 1 页 说明书 19 页 附图 1 页

[54] 发明名称 一种药物组合物及其在制备用于治疗糖尿病中的应用

[57] 摘要

本发明公开了一种药物组合物，该组合物含有 5mg～60mg 吡格列酮或其药学上可接受的盐，和不超过 3000mg 的二甲双胍或其药学上可接受的盐及一种或一种以上的药学上可接受的载体。 通过制成不同配比的复方制剂，改善服药方式，减少了服药次数，每日一次，方便患者长期服用。 本发明同时也公开了该组合物在制备用于治疗和/或预防糖尿病、与糖尿病有关的疾病及其某些并发症中的应用。

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1、一种含有 吡格列酮或其药学上可接受的盐，和二甲双胍或其药学上可接受的盐的药物组合，在制备用于治疗和／或预防糖尿病、与糖尿病有关的疾病及其某些并发症中的应用，其中，吡格列酮或其药学上可接受的盐的用量为 5~60mg，二甲双胍或其药学上可接受的盐的用量为不超过 3000mg。

2、根据权利要求 1 的应用，其中，所述的药学上可接受的盐，分别为盐酸吡格列酮和盐酸二甲双胍。

3、根据权利要求 1 的应用，其中，吡格列酮或其药用盐的用量为 5～15、15～30、30～45、45～60mg。

4、根据权利要求 1 的应用，其中，吡格列酮或其药用盐的用量为 5mg、15mg、30mg、45mg、60mg。

5、根据权利要求 1-4 中任一项的应用，其中，二甲双胍或其药用盐的用量为 250mg、750mg 或 1000mg。

6、一种药物组合物，该组合物含有 5～60mg 吡格列酮或其药用盐和不超过 3000mg 二甲双胍或其药用盐，及一种或一种以上的药学上可接受的载体。

7、根据权利要求 6 的组合物，该组合物中含有 5～15、15～30、30～45、45～60mg 吡格列酮或其药用盐。

8、根据权利要求 6 的组合物，该组合物含有 5mg、15mg、30mg、45mg、60mg。吡格列酮或其药用盐。

9、根据权利要求 6-8 中任一项组合物，该组合物中含有 250mg、750mg 或 1000mg 二甲双胍或其药用盐。

10、一种药物组合物的制备方法，其特征在于，可制成含 5～60mg 速释吡格列酮或其药用盐和不超过 3000mg 缓释二甲双胍或其药用盐的双层片，或可制成内层为不超过 3000mg 缓释二甲双胍或其药用盐，外层为含 5～60mg 速释的吡格列酮或其药用盐的双层片。

一种药物组合物及其在制备用于治疗糖尿病中的应用

技术领域

本发明属于治疗糖尿病药物领域，更具体地说是涉及一种吡格列酮和二甲双胍的药物组合物，以及该组合物制备在用于治疗和/或预防糖尿病、与糖尿病有关的疾病及其某些并发症中的应用。

背景技术

糖尿病是一组由遗传和环境因素相互作用而引起的临床综合症。据流行病学调查估计目前全球糖尿病患者总数已逾一亿，其中 90%左右为Ⅱ型糖尿病，其发病机理为胰岛素抵抗为主，伴有胰岛素分泌缺陷或胰岛素分泌缺陷为主，伴胰岛素抵抗和肝脏葡萄糖产生增加。Ⅱ型糖尿病患者常伴有肥胖、高血压、高脂血症、脂肪肝及冠心病等疾病。

美国专利第 3174901 号公开了双胍类抗高血糖药二甲双胍，其控制血糖的辅助机制是抑制肝脏产生葡萄糖和增加外周摄取葡萄糖，由此降低胰岛素抗性。

日本专利昭 61-267580，欧洲专利 Ep193256，美国专利 US4687777 公开了噻唑烷二酮类胰岛素增敏剂吡格列酮的抗高血糖和调节脂代谢作用，其作用机制与胰岛素存在有关，可减少外周组织和肝脏的胰岛素抗性，增加依赖胰岛素的葡萄糖处理，并减少肝糖的输出。下列文献公开了吡格列酮与二甲双胍联合应用的实例：(1) Einhorn D 等 Pioglitazone hydrochloride in combination with metformin in the treatment of type 2 diabetes mellitus: a randomized , placebo-controlled study. Clin Ther.2000 Dec;22(12):1395-409.公开了在单独使用二甲双胍血糖控制不佳时，与盐酸吡格列酮联合应用的治疗效果和耐受性。

(2) Suzuki M 等 Effects of combined pioglitazone and metformin on diabetes and

obesity in Wistar fatty rats. Clin Exp Pharmacol Physiol. 2002 Apr;29(4):269-74. 公开了盐酸吡格列酮和盐酸二甲双胍联合应用，对高血糖、高甘油酯血、高酮血的 Wistar 肥胖大鼠的治疗效果。

(3) 潘长玉 等在《盐酸二甲双胍治疗 II 型糖尿病的有效性和安全性的多中心临床研究》中公开了盐酸吡格列酮与磺脲类或双胍类联合应用时降糖效果和安全性。

吡格列酮是一种噻唑烷酮类胰岛素抵抗改善剂，降低胰岛素抵抗，保护 β 细胞功能，能有效治疗非胰岛素依赖性糖尿病肥胖患者的糖、脂异常。二甲双胍现已被广泛接受为轻、中度型糖尿病患者特别是肥胖患者的首选抗高血糖药物。甚至对于 II 型糖尿病的中间阶段 -IGT（葡萄糖耐量减低），亦有干预作用，可阻止或延缓由 IGT 状态进入糖尿病阶段。

胰岛素抵抗是 II 型糖尿病初始阶段的主要缺陷。胰岛素抵抗贯穿于 II 型糖尿病的发生、发展全过程，而噻唑烷二酮类盐酸吡格列酮与二甲双胍皆具降低胰岛素抵抗效果，但二者的作用机制不同，二者的作用部位也有差别， 吡格列酮主要促进外周组织(骨骼肌)摄取葡萄糖，可用于 II 型糖尿病的胰岛素耐受性，而二甲双胍主要抑制肝葡萄糖输出，故二者合用，作用集中在代谢缺陷，抗糖尿病效果可加强，有助于较单独使用二甲双胍达到更好的控制血糖。

发明内容

现在，有令人惊奇的现实表明，吡格列酮与二甲双胍的联合药物形式可提供特别有益的血糖控制作用而没有观察到副作用，观察到的协同作用在于低血糖的显著改善，因此这种联合药物形式特别可用于治疗糖尿病，尤其是 II 型糖病和与糖尿病有关的疾病。

因此，本发明提供了一种哺乳动物如人的糖尿病的治疗方法，该方法包含给予需要这种治疗的哺乳动物有效、无毒且药学上可接受量的胰岛素增敏剂如吡格列酮或其药学上可接受的盐，和双胍类抗高血糖剂如二甲双胍或其药学上可接受的盐的药物组合物，其中，吡格列酮或其药学上可接受的盐的用量为 5-60mg，二甲双胍或其药学上可接受的盐的用量为不超过 3000mg。

应当理解，吡格列酮和二甲双胍是分别以其药学上可接受的形式做为适当的相关药物

活性剂给药的，包括其药学上可接受的衍生物如药学上可接受的盐、酯和溶剂化物。应理解，本发明包括活性剂本身的所有药学上可接受的形式。二甲双胍的合适的药学上可接受的形式是酸加成盐，如盐酸盐、乙酸盐、苯甲酸盐、甲磺酸盐、马来酸盐等，然而，优选使用二甲双胍本身或其盐酸盐。参照 US3174901 方法通过二甲双胍与相应的酸反应得到二甲双胍的可药用盐。

吡格列酮合适的药学上可接受的盐包括盐酸盐、甲酸盐、富马酸盐、乙酸盐、苯甲酸盐、甲磺酸盐、硫酸盐、马来酸盐等，然而，优选使用吡格列酮本身或其盐酸盐。参照 EP193256 方法可制备吡格列酮的可药用盐。

在一个特定方面，该方法包含给予 5～60mg 吡格列酮或盐酸吡格列酮，尤其是当每天给药时。

特别是，该方法包含每天给予 5～15、15～30、30～45、45～60mg 吡格列酮或盐酸吡格列酮。

特别是，该方法包含每天给予 5～15mg 吡格列酮或盐酸吡格列酮，尤其是当每天给药时。

特别是，该方法包含每天给予 15～30mg 吡格列酮或盐酸吡格列酮，尤其是当每天给药时。

特别是，该方法包含每天给予 30～45mg 吡格列酮或盐酸吡格列酮，尤其是当每天给药时。

特别是，该方法包含每天给予 45～60mg 吡格列酮或盐酸吡格列酮，尤其是当每天给药时。

优选的是，该方法包含每天给予 5mg 吡格列酮或盐酸吡格列酮，尤其是当每天给药时。

优选的是，该方法包含每天给予 10mg 吡格列酮或盐酸吡格列酮，尤其是当每天给药时。

优选的是，该方法包含每天给予 15mg 吡格列酮或盐酸吡格列酮，尤其是当每天给药时。

优选的是，该方法包含每天给予 30mg 吡格列酮或盐酸吡格列酮，尤其是当每天给药时。

优选的是，该方法包含每天给予 45mg 吡格列酮或盐酸吡格列酮，尤其是当每天给药时。

优选的是，该方法包含每天给予 60mg 吡格列酮或盐酸吡格列酮，尤其是当每天给药时。

在一个特定方面，该方法包含给予不超过 3000mg 的二甲双胍或盐酸二甲双胍，尤其是当每天给药时。特别优选的是，二甲双胍或盐酸二甲双胍的用量为 250mg、750mg、1000mg，尤其是当每天给药时。

本发明解决了糖尿病患者需要长期多次服药的烦恼，通过制成不同配比的复方制剂，改善服药方式，减少了服药次数，每日一次，方便患者长期服用。

另一方面，本发明提供了一种吡格列酮与二甲双胍在制备用于治疗和／或预防糖尿病、与糖尿病有关的疾病，及其某些并发症中的应用，特别是，治疗糖尿病尤其是II型糖尿病和与糖尿病有关的疾病的方法中的应用。该方法包含将吡格列酮与二甲双胍同时给药。同时给药包括给予吡格列酮与二甲双胍的制剂，或者将每种活性剂的单独制剂基本上同时给药。

经大量的临床研究已证实，II型糖尿病诊断后 3 年内若只用一种药治疗，葡萄糖控制进展性下降，有互补作用的两药联合治疗经常被用来得到最大治疗效应和减小副作用。吡格列酮与二甲双胍两药联合应用不仅有效地控制血糖、降低胰岛素抵抗、保护β细胞功能，同时降低了低血糖的发生率，起到了协同作用。两药联合应用可延缓和阻止疾病的发展，预防糖尿病的长期并发症，如心脏病、失明、截肢和肾衰竭。

本文中使用的术语“与糖尿病有关的疾病”包括与前驱糖尿病状态有关的那些疾病、与糖尿病自身有关的疾病和与糖尿病有关的并发症。

本文中使用的术语“与前驱糖尿病状态有关的那些疾病”包括诸如胰岛素抵抗疾病，包括遗传性胰岛素抵抗、葡萄糖耐量减弱和高胰岛素血。

本文中使用的术语“与糖尿病自身有关的疾病”包括高血糖，胰岛素抵抗，包括后天胰岛素抵抗和肥胖。其他与糖尿病有关的疾病包括高血压和心血管疾病，尤其是动脉粥样硬化和与胰岛素有关的疾病。与胰岛素有关的疾病包括多囊性卵巢综合征和类固醇诱导的胰岛素抵抗和妊娠糖尿病。

“与糖尿病有关的并发症”包括肾脏疾病，尤其是与II型糖尿病有关的肾脏疾病，神经病和视网膜病。

与 II型糖尿病有关的肾脏疾病包括肾病，肾小球肾炎，肾小球硬化症，肾病综合征，高血压性肾硬化和晚期肾脏疾病。

本文中所用的术语“药学上可接受的”包含任何兽医用途：例如术语“药学上可接受

的”包含兽医学上可接受的化合物。

通过本发明治疗提供的特别有益的血糖控制作用，指示为相对于对照的协同作用，该对照预期为单独的活性药剂的作用总和。

在一个优选的方面，当根据本发明治疗而使用时，所用的各种活性剂的剂量水平将小于达到单纯加和的血糖控制作用可能需要的剂量。

再一方面，本发明提供了一种含有吡格列酮与二甲双胍的药物组合物。该组合物含有5～60mg 吡格列酮或盐酸吡格列酮和不超过 3000mg，二甲双胍或盐酸二甲双胍及一种或一种以上的药学上可接受的载体。

通常该组合物适于口服给药，但是，它们也适合其他的给药方式，例如胃肠外给药、舌下给药或经皮给药。

为了达到给药的一致性，本发明组合物优选为单剂形式。

用于口服给药的单剂表示形式可以是片剂和胶囊，可含有以下赋性剂诸如填充剂，乳糖、蔗糖、淀粉、微晶纤维素、山梨醇、磷酸钙；粘合剂，例如糖浆、明胶、羟丙基甲基纤维素、聚乙烯吡咯烷酮、淀粉、糊精；崩解剂，例如微晶纤维素、羧甲基淀粉钠、羧甲基纤维素钠、交联聚乙烯吡咯烷酮；润滑剂，例如硬脂酸镁；高分子骨架材料，例如羟丙基甲基纤维素、羟丙基纤维素、乙基纤维素、巴西棕榈蜡、氢化植物油、丙烯酸树脂；成膜材料，例如羟丙基甲基纤维素、聚乙烯吡咯烷酮、丙烯酸树脂等。

本发明优选的药物组合物的制备方法，可制成含 5～60mg 速释吡格列酮或其药用盐和不超过 3000mg 缓释二甲双胍或其药用盐的上下双层片，或可制成内层为不超过 3000mg 缓释二甲双胍或其药用盐，外层为含 5～60mg 速释的吡格列酮或其药用盐的双层片。

本发明中二甲双胍或其药用盐制成每天只需服用一次的缓释片，能在体内缓慢释放，维持血药浓度平稳，半衰期延长，安全、高效、低毒、服用方便，副作用和配伍禁忌较少，并方便与吡格列酮或其药用盐制成不同配比的复方制剂，且病人服用方便，不易漏服，增加了用药的顺从型。

这些组合物优选以与相关日剂量适宜的量制成单位剂型。

合适的吡格列酮的单位剂量包含5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、30、31、32、33、34、35、36、37、38、39、40、41、42、43、44、45mg吡格列酮。

本发明的组合物可以每天给药1～3次，但优选每天给药1或2次。

吡格列酮特定剂量是5mg/天，10mg/天，15mg/天，30mg/天，45mg/天、60mg/天。

二甲双胍合适的剂量包括每天不超过3000mg，优选以250mg、500mg、1000mg、1500mg或2000mg的单位剂量给药，二甲双胍剂量的一个例子是每次1000mg、每日一次。

吡格列酮和二甲双胍合适的单位剂量还包括这些化合物的已知剂量，如参考书中国药典、美国药典、英国药典、欧洲药典及PHYSICINS' DESK REFERENCE中描述或提到的。

以下通过药理的急性、毒性实验，药代动力学实验，药效学实验进一步阐述本发明。

一、复方二甲双胍/吡格列酮急性毒性试验：

1、试验目的：

观察复方二甲双胍/吡格列酮不同配比（500:7.5、500:15、500:30）单次口服给药后小鼠产生的急性毒性反应及死亡分布情况，计算LD_{50}值。为药效学试验、多次反复给药的毒性试验的复方配比和剂量设计及临床安全性提供参考。

2、实验材料：

（1）实验动物

昆明种小鼠，雌雄各半，体重18-22g。实验动物设施：二级；合格证号：津实动设施准第012号；实验动物合格证：W-J津实动质R准字第001号。实验环境和条件：室温22±4℃，湿度60±20%。中央空调自动通风。光照12小时。自由摄食和饮用自来水。每日换水一次。

（2）实验药物

复方二甲双胍/吡格列酮，药物用1%CMC配制成100mg/ml的混悬药液。

3、实验方法和结果：

（1）实验方法：

昆明种小鼠 50 只，雌雄各半，按性别随机分为 5 组，分别为 5000、4000、3200、2560、2048mg/kg 五个剂量组。动物禁食 6 小时给药。将药物用 1%CMC 混悬配制成 100mg/ml 的药液，采用等浓度不等体积的方式给药，给药体积分别为 0.50、0.40、0.32、0.26、0.20ml/10g。药后观察动物的毒性反应及其发生时间，死亡情况及时间，死亡动物进行解剖学检查，必要时进行组织学检查；存活动物连续观察 14 天，在试验的 0、1、3、7、14 天称体重，在第 14 天对部分存活动物进行解剖观察。统计动物死亡情况，计算半数致死量和动物体重的变化。

（2）实验结果：

复方二甲双胍/吡格列酮（500:7.5）口服给药急性毒性试验：

口服给予复方二甲双胍/吡格列酮(500:7.5)，药后 10-30 分钟动物出现活动减少，部分动物出现闭眼（1-6/10）；药后 1 小时，5只动物出现腹泻；药后 2 小时，10 只动物出现腹泻。毒性反应发生的时间、动物数及严重程度与给药剂量呈正相关。动物死亡最早出现在药后 5 小时，所有动物死亡均发生在药后 18 小时内。对死亡动物剖检，可见部分动物（6 只）轻度肺出血，其它脏器未见任何明显病变。药后 18 小时，所有存活动物基本恢复正常活动。存活动物在 14 天的观察期内未见死亡，动物体重增长未受明显影响。第 14 天取部分存活动物剖检未见明显病变。小鼠口服给药的半数致死量为 3137.3mg/kg。(结果见表 1、2)。

表 1.小鼠单次口服给予复方二甲双胍/吡格列酮（500:7.5）对存活动物体重的影响(g)

剂量 (mg/kg)	0d	1d	3d	、7d	14d
5000	19.8±0.9				
4000	19.9±1.0	20.8±2.5	23.0±2.8	26.5±3.5	29.3±3.9
3200	19.8±1.1	21.0±1.2	23.4±1.5	25.6±1.7	28.8±1.8

2560	19.9±1.0	20.6±1.1	22.3±1.2	24.8±1.6	28.6±2.3
2048	19.7±1.1	20.9±1.4	23.1±1.9	26.1±2.2	29.2±2.7

注：0d 动物体重为禁食后体重，动物数为各组存活动物数，见表 2。

表 2.小鼠单次口服给予复方二甲双胍/吡格列酮（500:7.5）LD_{50}测定结果

剂量 (mg/kg)	动物数 (只)	死亡只数(♂)	死亡只数(♀)	死亡总数(只)	死亡率 (%)	LD_{50}(mg/kg) (95%可信限)
5000	10	5	5	10	100	3137.3 (2834.9-3472.1)
4000	10	3	5	8	80	
3200	10	3	3	6	60	
2560	10	1	1	2	20	
2048	10	0	0	0	0	

复方二甲双胍/吡格列酮（500:15）口服给药急性毒性试验：

口服给予复方二甲双胍/吡格列酮(500:15)，药后 10-30 分钟动物出现活动减少，部分动物出现闭眼（1-4/10）；药后 1 小时，3 只动物出现腹泻，1 只动物后肢无力，步态不稳；药后 2 小时，9 只动物出现腹泻，1 只动物濒死。毒性反应发生的时间、动物数及严重程度与给药剂量呈正相关。动物死亡最早出现在药后 4 小时，所有动物死亡均发生在药后 18 小时内。对死亡动物剖检，可见部分动物（4 只）轻度肺出血，其它脏器未见任何明显病变。药后 18 小时，所有存活动物基本恢复正常活动。存活动物在 14 天的观察期内未见死亡，动物体重增长未受明显影响。第 14 天取部分存活动物剖检未见明显病变。小鼠口服给药的半数致死量为 3348.8mg/kg。(结果见表 3、4)。

表 3.小鼠单次口服给予复方二甲双胍/吡格列酮（500:15）对存活动物体重的影响(g)

剂量 (mg/kg)	0d	1d	3d	7d	14d
5000	19.7±0.9				
4000	19.6±1.1	20.3±1.0	22.3±1.0	26.0±1.3	29.0±2.6
3200	19.5±1.0	21.1±1.3	23.4±1.1	26.8±1.9	29.7±2.5

2560	19.7±1.0	21.2±1.2	22.8±1.7	26.2±1.8	29.8±2.0
2048	19.7±1.0	21.1±1.1	23.4±1.3	26.7±1.6	29.8±2.3

注：0d 动物体重为禁食后体重，动物数为各组存活动物数，见表 4。

表 4. 小鼠单次口服给予复方二甲双胍/吡格列酮（500: 15）LD_{50}测定结果

剂量 (mg/kg)	动物数 (只)	死亡只数(♂)	死亡只数(♀)	死亡总数(只)	死亡率 (%)	LD_{50}(mg/kg) (95%可信限)
5000	10	5	5	10	100	3348.8 (3029.6-3701.7)
4000	10	3	4	7	70	
3200	10	3	2	5	50	
2560	10	0	1	1	10	
2048	10	0	0	0	0	

复方二甲双胍/吡格列酮（500: 30）口服给药急性毒性试验：

口服给予复方二甲双胍/吡格列酮(500: 30)，药后 10-30 分钟动物活动减少，部分动物闭眼（1-4/10）；药后 1 小时，4 只动物腹泻；药后 2 小时，1 只动物步态不稳，7 只动物腹泻。毒性反应发生的时间、动物数及严重程度与给药剂量呈正相关。动物死亡最早出现在药后 5 小时，所有动物死亡均发生在药后 18 小时内。对死亡动物剖检，可见部分动物（10 只）轻度肺出血，其它脏器未见任何明显病变。药后 18 小时，所有存活动物基本恢复正常活动。存活动物在 14 天的观察期内未见死亡，动物体重增长未受明显影响。第 14 天取部分存活动物剖检未见明显病变。小鼠口服给药的半数致死量为 3726.7mg/kg。(结果见表 5、6)。

表 5. 小鼠单次口服给予复方二甲双胍/吡格列酮（500: 30）对存活动物体重的影响(g)

剂量 (mg/kg)	0d	1d	3d	7d	14d
5000	20.3±1.0				
4000	20.2±1.1	21.3±1.2	22.8±1.2	25.2±1.3	28.8±1.8
3200	20.4±0.9	21.9±1.6	23.5±2.0	26.2±2.6	28.5±2.4

2560	20.3±0.9	22.0±0.5	24.1±1.7	26.6±2.3	29.7±2.6
2048	20.2±1.1	21.9±0.9	23.8±1.0	26.7±1.4	29.6±2.5

注：0d 动物体重为禁食后体重，动物数为各组存活动物数，见表 6。

表 6. 小鼠单次口服给予复方二甲双胍/吡格列酮（500: 30）LD_{50} 测定结果

剂量 (mg/kg)	动物数 (只)	死亡只数(♂)	死亡只数(♀)	死亡总数(只)	死亡率 (%)	LD_{50}(mg/kg) (95%可信限)
5000	10	5	5	10	100	3726.7 (3338.6-4159.8)
4000	10	3	1	4	40	
3200	10	2	1	3	30	
2560	10	0	1	1	10	
2048	10	0	0	0	0	

4、结论：

小鼠单次口服给予三种配比的复方二甲双胍/吡格列酮出现的毒性反应基本一致，给药后 10-30 分钟部分动物活动减少、闭眼，药后 1 小时部分动物腹泻、步态不稳，毒性反应发生的动物数及严重程度与给药剂量呈正相关。动物死亡最早出现在药后 4-5 小时，所有动物死亡均发生在药后 18 小时内。对死亡动物剖检，可见部分动物轻度肺出血，其它脏器未见任何明显病变。药后 18 小时，所有存活动物基本恢复正常活动。存活动物在 14 天的观察期内未见死亡，动物体重增长未受明显影响。第 14 天取部分存活动物剖检未见明显病变。小鼠口服给予复方二甲双胍/吡格列酮不同配比（500: 7.5、500: 15、500: 30）的半数致死量分别为 3137.3、3348.8、3726.7mg/kg。

二、复方二甲双胍/吡格列酮降糖的药代动力学研究：

复方降糖是双胍类降糖药二甲双胍和噻唑烷酮类降糖药吡格列酮的复方制剂，简称复方降糖（500: 30, w/w），由于作用机理不同，配伍以期达到更佳的降糖效果。本试验旨在通过大鼠的吸收试验阐明复方制剂与单方制剂之间吸收的异同。

1、材料

(1) 药物:

二甲双胍: 临用前以水溶解成 15mg/ml, 给药容量为 1ml/100g 体重, 相当于 150mg/kg。

吡格列酮:临用前以 1%CMCNa 混悬成 0.9mg/ml,给药容量为 1ml/100g 体重,相当于 9mg/kg。

复方降糖: 临用前以 1%CMCNa 混悬成 15mg 二甲双胍和 0.9mg 吡格列酮/ml, 给药容量为 1ml/100g 体重, 相当于 150mg/kg 二甲双胍和 9mg/kg 吡格列酮。

(2) 试剂:

甲醇: 优级纯, 天津市康科德科技有限公司产品, 批号 031204。磷酸二氢钾: AR, 北京红星化工厂产品, 批号 851011-1。B7: 天津市化学试剂二厂产品。乙腈: AR, 天津市康科德科技有限公司产品, 批号 031015。乙酸钠: 天津石英钟厂霸州市化工分厂产品, 批号 980303。

(3) 仪 器:

NL-200TPA 分析天平: 日本岛津公司。

TGL-16C 高速台式离心机: 上海安亭科学仪器厂。

HPLC: WATERS 515 泵; 717 自动进样器; RAININ 紫外检测器;

ANASTAR 色谱数据工作站。

(4) 动物:

健康 Wistar 大鼠, 雌性, 体重约 210g, 实验动物设施合格证"津实验动物设施准第 013 号"由天津市实验动物管理委员会颁发, 符合一级标准。正常饲养三天后供试。

2、方法:

(1) 样品采集与处理:

健康 Wistar 大鼠 12 只,雌性,禁食 16 小时,按体重平均分为三组,即吡格列酮 9mg/kg 组, 二甲双胍 150mg/kg 组和复方降糖组。于早晨 8: 00 分别口服灌胃上述药物, 于药后 0.33, 0.66, 1.0, 1.5, 2.0, 4.0, 6.0, 12.0, 24.0 和 36.0 小时分别眼眶采血 0.5ml, 离心分离血清。

定量吸取二甲双胍组和复方降糖组动物的血清 50μ 1, 加入等体积 10%高氯酸, 充分振摇

沉淀蛋白，离心，上清液 20μ 1 进样，HPLC 分析。用空白血清复管操作配制标准血清样品，浓度分别为 0，0.5，1，2，5，10 和 20μ g/ml，作为标准曲线，处理方法同上。

定量吸取吡格列酮组和复方降糖组动物的血清 150μ 1，加入 1ml 二氯甲烷，充分振摇，离心，取 800μ 1 下层有机相置于另一个离心管中，空气吹干，75μ 1 流动相复溶，离心 20 μ 1 进样，HPLC 分析。用空白血清复管操作配制标准血清样品，浓度分别为 0，0.1，0.5，1，5 和 10μ g/ml，作为标准曲线，处理方法同上。

（2）色谱条件：

二甲双胍：固定相： C_{18} ODS 柱，4.6×250mm，10μ ，柱号 22I25117

流动相：甲醇：0.005M 磷酸二氢钾（pH2.5）=10：90

柱温：40℃

UV 检测：233nm

吡格列酮：固定相： C_{18} ODS 柱，4.6×100mm，5μ ，柱号 22K10040

流动相：乙腈：0.1M 乙酸钠（pH4.5）=39：61

柱温：30℃

UV 检测：269nm

（3） 结果：

二甲双胍：

血清标准曲线（0～20μ g/ml）的线性方程是：C=0.0000353A + 0.10355（r = 0.9992）。回收率为 95.04%。大鼠口服二甲双胍和复方降糖后不同时间的血药浓度见表 7，药时曲线见图 1；平均达峰时间分别为 1.1 和 1.0 小时；峰浓度分别为 19.6 和 20.4μ g/ml；AUC 分别为 239.8 和 249.9μ g• h/ml。复方相对于单方的生物利用度为 101.7%。

表 7. 大鼠口服二甲双胍和复方降糖后不同时间的血药浓度

组别	经时血药浓度(μ g/ml)										
	0.33	0.67	1	1.5	2	4	6	8	12	24	36 h
单方二甲双胍											

1	13.5	16.3	19.3	19.3	16.1	8.3	8.2	6.7	6.0	3.1	3.5
2	12.2	15.1	24.6	20.0	16.9	9.3	5.5	5.4	10.4	13.1	2.3
3	14.5	13.7	14.5	18.4	13.7	10.8	5.8	5.2	5.6	4.6	2.8
4	13.6	15.3	16.3	14.0	14.9	7.2	5.1	4.9	4.7	5.4	1.9
mean	13.4	15.1	18.7	17.9	15.4	8.9	6.2	5.5	6.7	6.6	2.6
复方降糖											
1	12.6	19.6	27.2	21.6	14.9	14.7	8.5	7.3	6.8	6.6	2.5
2	12.5	15.6	16.3	15.1	15.9	10.2	6.9	6.5	6.5	5.7	2.9
3	11.6	18.7	19.1	16.1	16.2	10.7	7.3	6.3	5.3	4.3	2.6
4	15.9	17.9	19.1	15.4	18.8	10.3	8.0	6.8	6.7	6.8	3.4
mean	13.1	18.0	20.4	17.1	16.4	11.5	7.7	6.7	6.3	5.8	2.8

吡格列酮:

血清标准曲线（0～10μ g/ml）的线性方程是: C=0.0000310A－0.1488（r＝0.9988)。回收率为 71.7%。大鼠口服吡格列酮和复方降糖后不同时间的血药浓度见表 8，药时曲线见图 2；平均达峰时间分别为 4.0 和 3.9 小时；峰浓度分别为 6.8 和 5.3μ g/ml；AUC 分别为 80.4 和 85.0μ g• h/ml。复方相对于单方的生物利用度为 105.7%。

表 8. 大鼠口服吡格列酮和复方降糖后不同时间的血药浓度

组别	经时血药浓度(μ g/ml)										
	0.33	0.67	1	1.5	2	4	6	8	12	24	36 h
单方吡格列酮											
1	0.68	2.95	3.49	5.32	3.71	4.72	4.36	4.13	2.48	0.36	nd
2	5.84	10.81	8.09	10.73	7.60	7.32	5.90	5.12	3.25	0.66	0.34
3	3.51	2.84	3.62	3.96	4.85	4.47	4.93	4.07	3.10	0.69	0.42
4	0.81	2.36	3.00	3.23	3.74	4.54	6.06	6.14	3.57	0.39	nd
mean	2.71	4.74	4.55	5.81	4.97	5.26	5.31	4.87	3.10	0.52	0.38
复方降糖											
1	1.05	2.29	2.80	2.87	2.85	3.26	2.38	1.86	1.12	2.58	0.40
2	0.42	0.46	3.16	3.81	3.19	5.49	5.95	5.09	3.41	1.73	2.47

3	0.44	3.37	4.21	5.41	5.48	5.95	5.09	3.40	1.68	2.44	0.69
4	3.33	4.21	5.41	5.93	4.57	3.64	5.28	3.22	1.85	0.80	1.31
mean	1.31	2.58	3.90	4.51	4.02	4.59	4.67	3.39	2.01	1.89	1.22

3、结论:

大鼠口服给药单方二甲双胍和复方降糖后平均达峰时间分别为 1.1 和 1.0 小时; 峰浓度分别为 19.6 和 20.4μ g/ml; AUC 分别为 239.8 和 249.9μ g• h/ml。复方相对于单方的生物利用度为 101.7%。大鼠口服吡格列酮和复方降糖后的平均达峰时间分别为 4.0 和 3.9 小时; 峰浓度分别为 6.8 和 5.3μ g/ml; AUC 分别为 80.4 和 85.0μ g• h/ml。复方相对于单方的生物利用度为 105.7%。复方降糖中所包含的两种降糖药的大鼠体内吸收基本无干扰, 与单方无显著性差异。

附图说明

图 1 为大鼠口服给药二甲双胍和复方制剂后药时曲线;

图 2 为大鼠口服给药吡格列酮和复方制剂给药后药时曲线。

三、复方二甲双胍/吡格列酮药效学试验:

1. 实验材料

1.1 实验动物:

Wistar 大鼠, 体重 140-160g。实验动物合格证: W-J 津实动质 R 准字第 001 号。

1.2 实验环境及条件

实验动物设施二级, 合格证号: 津实动设施准第 012 号; 室温 22±4℃, 湿度 60±20%。中央空调自动通风。光照 12 小时。自由摄食和饮用自来水。每日换水一次。

1.3 药物:

盐酸二甲双胍; 盐酸吡格列酮。将两药研磨成粉末混匀后, 用 1%CMC 配制成混悬液。

1.4 试剂及仪器:

链脲佐菌素（Streptozotocin，STZ），Sigma，S-0130，进口分装，北京欣经科生物技术公司提供。规格：1g/瓶 。纯度：98%。

京都血糖仪（SUPER GLUCOCARD II）及检测试纸条，日本生产，北京麦邦生物工程技术公司提供。

胰岛素试剂盒：天津舒普生物工程技术公司提供。批号：06043-A

SUNRISE 遥控酶标仪，TECAN 产品。

2. 实验方法及结果

Wistar 大鼠，雄性，300 只，140g-160g，禁食 16hr，腹腔注射 30mg/kg 的 STZ（4℃冰浴中溶于 0.1mol/L 的 PH 值 4.4 的柠檬酸缓冲液，配后立即使用），每天 1 次，连续 3 次，给药 2 周后喂以高脂高糖饲料（基础饲料 55%，猪油 25%，蔗糖 20%），喂养 6 周后测大鼠 FBG（测前禁食 12h），挑选 FBG≥12.0mmol/L 的大鼠 64 只，随机分为 8 组，每组 8 只，分别设为模型对照组和复方 300:1.5 组、复方 300:3 组、复方 300:4.5 组、复方 300:6 组、复方 300:6.75 组、复方 300:9 组、复方 300:27 组。另取 8 只正常雄性 Wistar 大鼠（与上述大鼠同批领取，FBG≤5.0mmol/L）作为生理对照组。生理对照和模型对照组灌胃给予 1%CMC，7 个复方组依次灌胃给予 300mg:1.5mg/kg、300mg:3mg/kg、300mg: 4.5mg/kg、300mg:6mg/kg、300mg:6.75mg/kg、300mg:9mg/kg、300mg: 27mg/kg 的复方药物（盐酸二甲双胍：盐酸吡格列酮）。将受试药物用 1%CMC 配制成不同浓度的混悬液，每日上午 i.g 给药，给药体积均为 1ml/100g，生理对照和模型对照组灌胃给予等体积的 1%CMC。连续给药 21d，第 22d 早上 9: 00 用毛细玻璃管从眼底静脉丛取血（取血前禁食 12h）1 滴，用血糖检测仪测 FBG，另取血 1ml，离心取血清，按试剂盒说明方法采用 ELASE 法测空腹血清胰岛素（FINS）。计算：给药前后降糖绝对值{降糖绝对值=给药 21dFBG-给药前 FBG}、降糖百分率（降糖百分率=[(给药 21dFBG-给药前 FBG)/ 给药前 FBG×100%]）及胰岛素抵抗指数（IR）（IR= FBG×FINS/22.5）。各项数据以平均值±标准差表示，各组与模型对照组采用组间 t 检验进

行比较。结果：各个比例的复方药物均可不同程度的降低高血糖大鼠的 FBG，复方 300mg: 3mg/kg 以上剂量与模型对照组比较，降糖绝对值及降糖百分率有显著差异，且有一定的剂量相关性；各个比例的复方药物均可明显降低高血糖大鼠的 FINS 与 IR 水平。见表 9、10。

表 9 复方盐酸二甲双胍-吡格列酮对大鼠空腹血糖的影响

组别	动物数	给药前 FBG (mmol/L)	给药 21d FBG (mmol/L)	FBG 差值(21d-1d) (mmol/L)	给药 21d 降糖百分率 (%)
空白对照	8	3.7±0.5 ***	3.8±0.5 ***	0.1±0.4	2.7±11.7
模型对照	8	20.5±3.9	20.0±3.5	-0.5±1.3	-2.1±6.0
复方 300:1.5	8	20.3±5.2	17.9±3.9	-2.4±2.5	-10.7±10.1
复方 300:3	8	20.5±4.9	17.6±3.2	-2.9±2.4 *	-12.8±8.8 *
复方 300:4.5	8	20.4±4.3	17.7±4.3	-2.7±2.6	-13.1±11.5 *
复方 300:6	8	20.5±4.2	17.3±6.0	-3.2±2.8 *	-17.3±16.4 *
复方 300:6.75	8	20.3±4.0	16.4±3.0	-3.9±2.2 **	-18.7±8.5 ***
复方 300:9	8	20.5±5.5	15.9±4.3	-4.6±2.3 ***	-22.5±9.4 ***
复方 300:27	8	20.3±3.6	16.2±3.4	-4.1±2.5 **	-20.0±12.2 **

注：与模型对照组比较，*p<0.05,**p<0.01, ***p<0.001

表 10 复方盐酸二甲双胍-吡格列酮对大鼠空腹胰岛素及胰岛素抵抗的影响

组别	动物数	给药 21d FBG (mmol/L)	给药 21d FINS (mmol/L)	给药 21d 胰岛素抵抗指数(对数)
空白对照	8	3.8±0.5 ***	3.86±0.82 ***	-0.20±0.12 ***
模型对照	8	20.0±3.5	32.35±15.93	1.40±0.29
复方 300:1.5	8	17.9±3.9	8.80±4.63 **	0.79±0.25 ***
复方 300:3	8	17.6±3.2	6.53±4.00 **	0.65±0.22 ***
复方 300:4.5	8	17.7±4.3	12.68±9.63 **	0.87±0.31 **
复方 300:6	8	17.3±6.0	8.27±4.56 **	0.70±0.25 ***
复方 300:6.75	8	16.4±3.0	7.28±4.04 **	0.66±0.25 ***
复方 300:9	8	15.9±4.3	9.18±5.00 **	0.72±0.37 ***
复方 300:27	8	16.2±3.4	9.64±4.33 **	0.79±0.25 ***

注：与模型对照组比较，*p<0.05,**p<0.01, ***p<0.001

3. 实验结论

复方盐酸二甲双胍-吡格列酮（300mg: 1.5mg/kg、300mg: 3mg/kg、300mg: 4.5mg/kg、300mg: 6mg/kg、300mg: 6.75mg/kg、300mg: 9mg/kg、300mg: 27mg/kg）分别连续灌胃给药

21d，各个比例的复方药物均可不同程度的降低高血糖大鼠的 FBG、FINS 与 IR 水平，对 FINS 与 IR 的降低尤其明显。

具体实施方式

下面结合实施例对本发明做进一步的描述,但这些实施例并非对本发明的限制。为了更充分的解释本发明的实施，提供下述制剂实施例。制剂可以采用本发明中的任意一个组合物的形式。特选复方二甲双胍/吡格列酮不同配比（1）500:15（2）750:15（3）1000:15为代表。

制剂 1

上下双层片剂:

上层成分	用量/片	重量浓度(%)
盐酸吡格列酮	15 mg	12.1
微晶纤维素	55 mg	44.4
乳糖	45 mg	36.3
聚乙烯吡咯烷酮	3 mg	2.4
羧甲基淀粉钠	4.5 mg	3.6
硬脂酸镁	0.5 mg	0.4
滑石粉	1 mg	0.8

下层成分	用量/片	用量/片	用量/片
盐酸二甲双胍	500mg	750mg	1000mg
羟丙基甲基纤维素	190g	205mg	210mg
聚乙烯吡咯烷酮	14.0mg	19.1mg	24.2mg
硬脂酸镁	7.1mg	9.7mg	12.3mg

制备方法:

盐酸吡格列酮颗粒制备方法：将活性成分、乳糖、微晶纤维素过筛，并充分混合，用聚乙烯吡咯烷酮溶液与上述的粉混合，过筛，制得湿颗粒于 50-60℃干燥，将羧甲基淀粉

钠，硬脂酸镁和滑石粉预先过筛，然后加入到上述的颗粒中。

盐酸二甲双胍颗粒制备方法：将活性成分、羟丙基甲基纤维素过筛，并充分混合，用聚乙烯吡咯烷酮乙醇溶液与上述的粉混合，过筛，制得湿颗粒于 50-60℃干燥，加入硬脂酸镁到上述的颗粒中。

将上述颗粒置双层片压片机压片。

制剂 2

内外双层片剂：

内层成分	用量/片	用量/片	用量/片
盐酸二甲双胍	500mg	750mg	1000mg
羟丙基甲基纤维素	190g	205mg	210mg
聚乙烯吡咯烷酮	14.0mg	19.1mg	24.2mg
硬脂酸镁	7.1mg	9.7mg	12.3mg

外层成分	用量/片
盐酸吡格列酮	15mg
羟丙基甲基纤维素	43.6mg
聚乙二醇 400	4.5mg

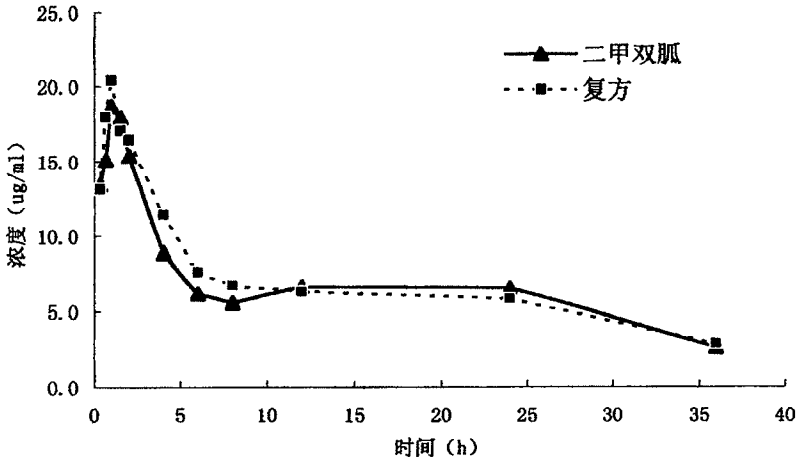
制备方法：

盐酸二甲双胍缓释片芯制备方法：将活性成分、羟丙基甲基纤维素过筛，并充分混合，用聚乙烯吡咯烷酮乙醇溶液与上述的粉混合，过筛，制得湿颗粒于 50-60℃干燥，加入硬脂酸镁到上述的颗粒中，压片。

盐酸吡格列酮包衣液：将盐酸吡格列酮与羟丙基甲基纤维素混合均匀，取适量聚乙二醇 400 加入水中溶解，搅拌下，缓缓加入上述混合物，使成混悬液，包衣液固体含量约为 9%。

混悬液过 80 目筛，适宜条件下包衣，控制增重量，使每片约含盐酸吡格列酮 15mg。

尽管本发明结合它的专门的实施例已做了详细的描述，但是很明显对本技术领域的熟练人来说仍能做出各种各样的变化和改进，都不会偏离本发明的精神实质和保护范围。


25.0
20.0
15.0
10.0
5.0
0.0
浓度（ug/ml）
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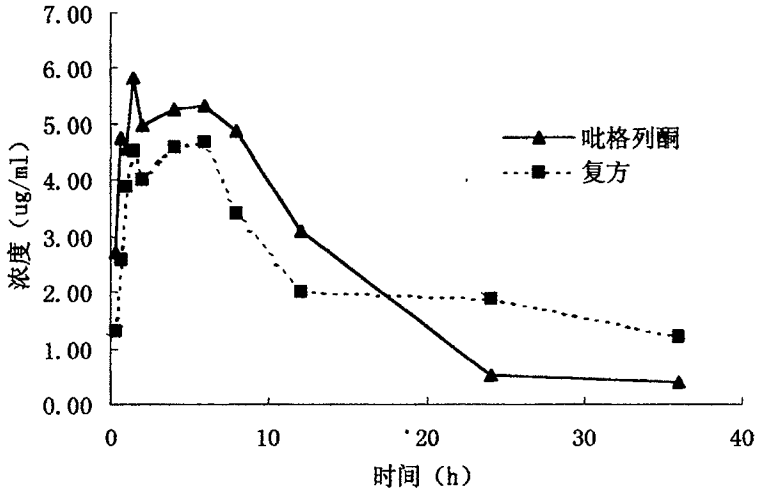
5
10
15
20
25
30
35
40
时间（h）
二甲双胍
复方

图 1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6.00
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4.00
3.00
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0
10
20
30
40
时间（h）
吡格列酮
复方

图 2

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Claims - 1 page, Prospectus - 19 pages, Drawings - 1 page

[54] **Name of Invention:** **A pharmaceutical composition and its application in the preparation of medicines used in the treatment of diabetes**

[57] **Abstract:**

The present invention discloses a pharmaceutical composition, wherein the composition contains 5 ~ 60 mg of Pioglitazone or its pharmaceutically acceptable salt and not more than 3,000 mg of Metformin or its pharmaceutically acceptable salt and one or more pharmaceutically acceptable carriers. Through preparing preparations with different proportions, manner of medicine intake is improved and medicine intake frequency is reduced to once a day for the convenience of patients' long-term medicine intake. The present invention also discloses application of the composition in the preparation of medicines used in the treatment and/or prevention of diabetes, diabetes related illnesses and certain complications thereof.

A pharmaceutical composition and its application in the preparation of medicines used in the treatment of diabetes

1. An application of a pharmaceutical composition in the preparation of medicines used in the treatment and/or prevention of diabetes, diabetes related illnesses and certain complications thereof, the pharmaceutical composition containing Pioglitazone or its pharmaceutically acceptable salt and Metformin or its pharmaceutically acceptable salt, wherein the dosage of Pioglitazone or its pharmaceutically acceptable salt is 5 ~ 60 mg, and the dosage of Metformin or its pharmaceutically acceptable salt is not more than 3,000 mg.

2. The application as defined in Claim 1, wherein the pharmaceutically acceptable salts are Pioglitazone Hydrochloride and Metformin Hydrochloride respectively.

3. The application as defined in Claim 1, wherein the dosage of Pioglitazone or its pharmaceutical salt is 5 ~ 15 mg, 15 ~ 30 mg, 30 ~ 45 mg, or 45 ~ 50 mg.

4. The application as defined in Claim 1, wherein the dosage of Pioglitazone or its pharmaceutical salt is 5 mg, 15 mg, 30 mg, 45 mg, or 60 mg.

5. The application as defined in any of Claims 1, 2, 3 and 4, wherein the dosage of Metformin or its pharmaceutical salt is 250 mg, 750 mg, or 1,000 mg.

6. A pharmaceutical composition, the composition containing 5 ~ 60 mg of Pioglitazone or its pharmaceutically acceptable salt and not more than 3,000 mg of Metformin or its pharmaceutically acceptable salt and one or more pharmaceutically acceptable carriers.

7. The pharmaceutical composition as defined in Claim 6, the composition containing 5 ~ 15 mg, 15 ~ 30 mg, 30 ~ 45 mg, or 45 ~ 50 mg of Pioglitazone or its pharmaceutical salt.

8. The pharmaceutical composition as defined in Claim 6, the composition containing 5 mg, 15 mg, 30 mg, 45 mg, or 60 mg of Pioglitazone or its pharmaceutical salt.

9. The pharmaceutical composition as defined in any of Claims 6 - 8, the composition containing 250 mg, 750 mg, or 1,000 mg of Metformin or its pharmaceutical salt.

10. A method for preparing a pharmaceutical composition, characterized in that the method is capable of preparing double-layer tablets containing 5 ~ 60 mg of repaid-release Pioglitazone or its pharmaceutical salt and not more than 3,000 mg of slow-release Metformin or its pharmaceutical salt.

A pharmaceutical composition and its application in the preparation of medicines used in the treatment of diabetes

FIELD OF THE DISCLOSURE

The present invention relates to the field of diabetes medicines and, more particularly, to a pharmaceutical composition of Pioglitazone and Metformin and an application of the pharmaceutical composition in the preparation of medicines used in the treatment and/or prevention of diabetes, diabetes related illnesses and certain complications thereof.

BACKGROUND

Diabetes is a group of clinical syndromes caused by interactions between genetic and environmental factors. According to epidemiological survey, it is estimated that the global diabetic patient population has exceeded 100 million, of which about 90% suffers from type 2 diabetes. The pathogenesis of type 2 diabetes in most cases mainly involves insulin resistance coupled with insulin secretory deficiency, or mainly involves insulin secretory deficiency coupled with insulin resistance and increase in hepatic glucose production. Type 2 diabetes patients often have the problems of obesity, high blood pressure, fatty liver, coronary heart disease, etc.

US Patent No. 3,174,901 discloses an anti-hyperglycemic drug Metformin, the auxiliary mechanism of which is to inhibit hepatic glucose production and to increase glucose ingestion by peripheral tissues, thereby reducing insulin resistance.

Japanese Patent No. Showa 61-267580 (JP-A-61-267580), European Patent No. EP193256 and US Patent No. 4,687,777 disclose the anti-hyperglycemic and lipid metabolism effects of a thiazolidinedione insulin sensitizing agent Pioglitazone. The working mechanism of Pioglitazone is related to the existence of insulin and may reduce the insulin resistance of peripheral tissues and that of liver and increase insulin-dependent glucose disposal, thereby reducing hepatic sugar output. The following literatures disclose implementation embodiments of combinative application of Pioglitazone and Metformin:

(1) Einhorn D. et al: Pioglitazone Hydrochloride in combination with Metformin in the treatment of type 2 diabetes mellitus: a randomized, placebo-controlled study – Clin Ther. 2000 Dec; 22(12): 1395-409 discloses the therapeutic effect and tolerability of combined application of Metformin and Pioglitazone Hydrochloride when blood sugar control is not satisfactory with the sole use of Metformin.

(2) Suzuki M. et al: Suzuki M. Effects of combined Pioglitazone and Metformin on diabetes and ...

... obesity in Wistar fatty rats - Clin Exp Pharmacol Physio 1. Apr 2002; 29(4): 269-74 discloses the therapeutic effect of combinative application of Pioglitazone Hydrochloride and Metformin Hydrochloride on hyperglycemic, hyperglyceridemic and hyperketonemic Wistar fatty rats.

(3) Pan Chang-yu et al: "Multi-center Clinical Trial of The Efficacy and Safety of Metformin Hydrochloride in The Treatment of Type 2 Diabetes" discloses the hypoglycemic effect and safety of combination application of Pioglitazone Hydrochloride with sulfonylurea drugs or biguanide drugs.

Pioglitazone is a thiazolidinone insulin resistance improving agent used to reduce insulin resistance and protect β-cell function, and it may effectively treat glycolipid abnormalities of non-insulin-dependent diabetic obese patients. Metformin has now been widely accepted as a first choice anti-hyperglycemic drug for mild to moderate diabetic patients, especially for obese patients. Pioglitazone even has intervention effects on the intermediate stage of type 2 diabetes - IGT (impaired glucose tolerance), and it may prevent or delay the advancement from IGT to severe diabetes.

Insulin resistance is the main deficiency at the initial stage of type 2 diabetes. Insulin resistance runs through the entire process of the occurrence and development of type 2 diabetes, and both the thiazolidinedione insulin Pioglitazone Hydrochloride and Metformin have reduction effect on insulin resistance by they have different working mechanisms and differ in action locations. Pioglitazone mainly enhances glucose ingestion of peripheral tissues (muscles) and may be used for improving insulin tolerance in the treatment of type 2 diabetes, while Metformin mainly inhibits hepatic glucose output. Combinative application of both is focused on metabolism deficiency and this may enhance anti-diabetic effects and help achieve better control of blood glucose level as compared to sole use of Metformin.

SUMMARY OF THE PRESENT INVENTION

Surprising facts have now indicated that the combined pharmaceutical form of Pioglitazone and Metformin provides extraordinarily beneficial blood glucose control effects and no adverse side effects have been observed. Concerted effect observed is the distinct improvement in hypoglycemia symptom, and this combined pharmaceutical form is particularly useful in the treatment of diabetes especially type 2 diabetes and diabetes related illnesses.

Therefore, the present invention provides a treatment method for diabetes in mammals such as human. The method comprises administering to the mammal needing this treatment a pharmaceutical composition of effective, non-toxic, pharmaceutically acceptable dosages of an insulin sensitizing agent such as Pioglitazone or its pharmaceutical acceptable salt, and a biguanide anti-hyperglycemic drug such as Metformin or its pharmaceutically acceptable salt, wherein the dosage of Pioglitazone or its pharmaceutically acceptable salt is 5 ~ 60 mg, and the dosage of Metformin or its pharmaceutically acceptable salt is not more than 3,000 mg.

It should be noted that Pioglitazone and Metformin are administered in their respective pharmaceutically acceptable forms as appropriate related active pharmaceutical agents, including ...

... their pharmaceutically acceptable derivatives such as their pharmaceutically acceptable salts, esters and solvates. It should be noted that the present invention includes all pharmaceutically acceptable forms of the active agents themselves. The appropriate pharmaceutically acceptable forms of Metformin are acid salts such as hydrochloride salt, acetate salt, benzoate salt, mesylate salt, maleate salt, etc. However, Metformin itself or its hydrochloride salt is preferably used. Refer to the method of US Patent No. 3,174,901 for obtaining pharmaceutical salts of Metformin through reactions between Metformin and the corresponding acids.

The appropriate pharmaceutically acceptable forms of Pioglitazone include hydrochloride salt, formate salt, fumarate salt, acetate salt, benzoate salt, mesylate salt, sulphate salt, maleate salt, etc. However, Pioglitazone itself or its hydrochloride salt is preferably used. Refer to the method of EP103256 for preparing pharmaceutical salts of Pioglitazone.

In one aspect, the method comprises administering 5 ~ 60 mg of Pioglitazone or Pioglitazone Hydrochloride, especially during daily administration.

In particular, the method comprises administering 5 ~ 15 mg, 15 ~ 30 mg, 30 ~ 45 mg, or 45 ~ 50 mg of Pioglitazone or Pioglitazone Hydrochloride.

In particular, the method comprises administering 5 ~ 15 mg of Pioglitazone or Pioglitazone Hydrochloride, especially during daily administration.

In particular, the method comprises administering 15 ~ 30 mg of Pioglitazone or Pioglitazone Hydrochloride, especially during daily administration.

In particular, the method comprises administering 30 ~ 45 mg of Pioglitazone or Pioglitazone Hydrochloride, especially during daily administration.

In particular, the method comprises administering 45 ~ 60 mg of Pioglitazone or Pioglitazone Hydrochloride, especially during daily administration.

Preferably, the method comprises administering 5 mg of Pioglitazone or Pioglitazone Hydrochloride, especially during daily administration.

Preferably, the method comprises administering 10 mg of Pioglitazone or Pioglitazone Hydrochloride, especially during daily administration.

Preferably, the method comprises administering 15 mg of Pioglitazone or Pioglitazone Hydrochloride, especially during daily administration.

Preferably, the method comprises administering 30 mg of Pioglitazone or Pioglitazone Hydrochloride, especially during daily administration.

Preferably, the method comprises administering 45 mg of Pioglitazone or Pioglitazone Hydrochloride, especially during daily administration.

Preferably, the method comprises administering 60 mg of Pioglitazone or Pioglitazone Hydrochloride, especially during daily administration.

In one aspect, the method comprises administering not more than 3,000 mg of Metformin or Metformin Hydrochloride, especially during daily administration. Particularly preferably, the dosage of Metformin or Metformin Hydrochloride is 250 mg, 750 mg, or 1,000 mg, especially during daily administration.

The present invention provides a solution for diabetes patients to overcome the trouble of having to frequently take medicines on a long term basis through preparing compound preparations with different proportions in order to improve medicine-taking mode and reduce frequency of medicine taking to once a day for the convenience of patients.

In another aspect, the present invention provides an application of the pharmaceutical composition of Pioglitazone and Metformin in the preparation of medicines used in the treatment and/or prevention of diabetes, diabetes related illnesses and certain complications thereof, particularly in the method of treatment of diabetes especially type 2 diabetes and diabetes related illnesses. The method comprises simultaneously administering Pioglitazone and Metformin. The simultaneous administration comprises administering preparation of Pioglitazone and Metformin or basically simultaneously administering single preparation of each active agent.

Through extensive clinical research it has been proven that if only one drug is administered within three years after diagnosis of type 2 diabetes, evolution of blood glucose control will be reduced, and combinative treatment by the two pharmaceuticals with mutually complementary effects is often carried out to achieve maximum therapeutic effect and to reduce adverse side effect. Combinative application of Pioglitazone and Metformin does not only effectively control blood glucose level, reduce insulin resistance and protect β-cell function, but also reduce the occurrence of hypoglycemia, thereby exerting their concerted effects. Combinative application of the two pharmaceuticals may delay and prevent development of the illnesses and prevent chronic complications of diabetes such as cardiopathy, ablepsy, amputation and renal failure.

The term "diabetes related illnesses" as used herein includes pre-diabetic state related illnesses, illnesses associated with diabetes itself, and diabetes related complications.

The term "pre-diabetic state related illnesses" as used herein includes insulin resistance symptoms, including inherited insulin resistance, impaired glucose tolerance and hyperinsulinemia.

The term "illnesses associated with diabetes itself" as used herein includes hyperglycemia and insulin resistance, including acquired insulin resistance, and pimelosis. Other illnesses related to diabetes itself include high blood pressure and cardiovascular diseases, especially atherosclerosis and insulin related diseases. Insulin related diseases include polycystic ovarian syndrome and steroid-induced insulin resistance and gestational diabetes.

The term "diabetes related complications" as used herein includes nephropathies, especially nephropathies associated with type 2 diabetes, neuropathies and retinal diseases.

Nephropathies associated with type 2 diabetes include nephropathy, glomerular nephritis, glomerular sclerosis, nephritic syndrome, hypertensive nephrosclerosis and late nephropathy.

The term "pharmaceutically acceptable" covers any veterinary usage, e.g. the term "pharmaceutically acceptable" covers ...

... any veterinally acceptable compounds.

Through extraordinarily beneficial control of blood glucose level provided by the therapeutic method of the present invention, concerted effect with respect to control is expressed, and the control is expected to be the total effect of the single active agents.

In a preferred aspect of the present invention, dosage levels of the various active agents used will be less than the dosage potentially required to achieve the simple sum of blood glucose control effect.

In yet another aspect, the present invention provides a pharmaceutical composition containing Pioglitazone and Metformin. The pharmaceutical composition contains 5 ~ 60 mg of Pioglitazone or Pioglitazone Hydrochloride and not more than 3,000 mg of Metformin or Metformin Hydrochloride and one or more pharmaceutical carriers.

The pharmaceutical composition is usually suitable for oral administration. However, it is suitable for other modes of administration, e.g. extra-astrointestinal administration, sublingual administration and percutaneous administration.

To achieve pharmaceutical administration consistency, single dose of the pharmaceutical composition of the present invention is preferred.

Single dosage used in oral administration may be in the form of tablets or capsules and contain excipients such as filling agents, lactose, sucrose, amylum, microcrystalline cellulose, sorbitol, and calcium phosphate; adhesive agents such as molasses, gelatin, hydroxypropyl methyl cellulose (HPMC), polyvinylpyrrolidone (PVP), and amylum, dextrin; disintegrating agents such as microcrystalline cellulose, hydroxymethyl starch sodium, and polyvinylpolypyrrolidone (PVPP); lubricating agent such as magnesium stearate; high molecular skeleton materials such as hydroxypropyl methyl cellulose (HPMC), hydroxypropyl cellulose (HPC), ethyl cellulose (EC), carnauba wax, hydrogenated vegetable oil, and acrylic acid resin; and film forming materials such as hydroxypropyl methyl cellulose (HPMC), polyvinylpyrrolidone (PVP), acrylic acid resin, etc.

The preferred method for preparation of the pharmaceutical composition of the present invention may prepare double-layer tablets with the top and bottom layers respectively containing 5 ~ 60 mg of repaid-release Pioglitazone or its pharmaceutical salt and not more than 3,000 mg of slow-release Metformin or its pharmaceutical salt, or double-layer tablets with the inner layer containing not more than 3,000 mg of slow-release Metformin or its pharmaceutical salt and with the outer layer containing 5 ~ 60 mg of repaid-release Pioglitazone or its pharmaceutical salt.

In the present invention, Metformin or its pharmaceutical salt is prepared as slow-release tablets to be taken once a day. The Metformin or its pharmaceutical salt is released slowly in the body to maintain stability of blood drug concentration. The Metformin or its pharmaceutical salt has an extended half-life and it is safe, highly effective, of low toxicity, and convenient for intake. It has less side effects and contraindicated combinations and is suitable for preparation of compound preparations with Pioglitazone or its pharmaceutical salt with different proportions. It is convenient for taking by patients, making them not prone to miss their doses. This enhances pharmaceutical compliance.

These pharmaceutical compositions are preferably prepared as unit doses with amounts suitable for the respective daily dosages.

Suitable unit doses of Pioglitazone include 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 11 mg, 12 mg, 13 mg, 14 mg, 15 mg, 16 mg, 17 mg, 18 mg, 19 mg, 20 mg, 21 mg, 22 mg, 23 mg, 24 mg, 25 mg, 26 mg, 27 mg, 28 mg, 29 mg, 30 mg, 31 mg, 32 mg, 33 mg, 34 mg, 35 mg, 26 mg, 37 mg, 38 mg, 39 mg, 40 mg, 41 mg, 42 mg, 43 mg, 44 mg and 45 mg Pioglitazone.

The pharmaceutical composition of the present invention may be administered 1 ~ 3 times a day, but preferably administered once or twice a day.

Specific doses of Pioglitazon are 5 mg per day, 10 mg per day, 15 mg per day, 30 mg per day, 45 mg per day and 60 mg per day.

Suitable doses of Metformin include not more than 3,000 mg per day, and are preferably administered with unit doses of 250 mg, 500 mg, 1,000 mg, 1,500 mg, or 2,000 mg. An example of Metformin dosage is 1,000 mg each time and once a day.

Unit doses of Pioglitazone and Metformin also include known doses of these compounds as described or mentioned in reference books such as Pharmacopoeia of the People's Republic of China, Pharmacopoeia of the United States of America, British Pharmacopoeia, European Pharmacopoeia and Physicians' Desk Reference.

The present invention is further illustrated by the following pharmacological acute toxicity experiments, pharmacokinetic experiments and pharmacodynamic experiments:

I. Acute Toxicity Experiment of Metformin / Pioglitazone Compound

1. Experiment Objectives

Observe the acute toxicity reaction and mortality distribution of mice after single oral administration of Metformin / Pioglitazone compound of different proportions (500: 7.5, 500: 15, 500: 30) and compute LD_{50} (Lethal Dosage, 50%) values. Provide reference information for the compound proportion and dosage design for pharmacodynamic experiments and toxicity experiments of repeated administration and the clinical safety thereof.

2. Experiment Materials

(1) Animals for Experiment

Kunming mice - 50% male and 50% female, weight ranging from 18 ~ 22 g. Laboratory animal facilities: Class 2; Certification Number: Jin Shi Dong Sheshi Zhun No. 012 (Tianjin Laboratory Animal Facilities Approval No. 012); Laboratory animal certification: W-J Jin Shi Dong Zhi R Zhun No. 001. Laboratory environment and conditions: Room temperature 22±4 °C, relative humidity 60±20%. Centralized air-conditioning with automatic ventilation. Illumination was provided for 12 hours. Food and piped water were self fed. Water was replaced once a day.

(2) Pharmaceuticals for Experiment

Metformin / Pioglitazone compound and pharmaceutical grade 1% carboxy methyl cellulose (CMC) were used to prepare 100 mg/ml medicinal suspension.

3. Experiment Method and Results

(1) Experiment Method

50 Kunming mice (50% male and 50% female) were randomly divided based on genders into 5 groups, namely the 5,000 mg/kg, 4,000 mg/kg, 3,200 mg/kg, 2,560 mg/kg, and 2,048 mg/kg dosage groups. The animals were made to fast before medicine administration. Medicine and 1% carboxy methyl cellulose (CMC) were used to prepare 100 mg/ml medicinal suspension, and medicine was administered in a mode with equal concentration but different volumes; and the volumes administered were 0.5 ml/10 g, 0.40 ml/10 g, 0.32 ml/10 g, 0.26 ml/10 g, and 0.20 ml/10 g.

(2) Experiment Results

Acute Toxicity Experiment of Metformin / Pioglitazone Compound (500: 7.5) Oral Administration:

Metformin / Pioglitazone compound (500: 7.5) was orally administered. 10 - 30 minutes after administration, reduction in animal activities was observed with some of the animals closing their eyes (1/10 to 6/10); one hour after administration, 5 animals demonstrated diarrhoea; and two hours after administration, 10 animals demonstrated diarrhoea. The time of occurrence of toxic reaction, the number of animals involved, and the degree of severity thereof appeared to be positively correlated with the dosage administered. Animal mortality first occurred 5 hours after administration and all animal mortalities occurred within 18 hours after administration. Animal anatomy indicated that some (6) of the animals had mild pneumonic hemorrhage, and no pathological lesions of organs were detected. 18 hours after administration, all surviving animals basically resumed normal activities. None of the surviving animals suffered mortality within the observation period of 14 days, and growth of animal weights was not distinctly affected. On the 14th day, some of the surviving animals were taken for anatomy and no pathological lesions were detected. LD_{50} (Lethal Dosage, 50%) of the oral administration to mice was 3,137.3 mg/kg. (Results are as shown in Table 1 and Table 2).

Table 1.
Effect of Single Oral Administration of Metformin / Pioglitazone Compound (500: 7.5) on Weight of Surviving Mice (g)

Dosage (mg/kg)	0th day	1st day	3rd day	7th day	14th day
5,000	19.8 ± 0.9	-	-	-	-
4,000	19.9 ± 1.0	20.8 ± 2.5	23.0 ± 2.8	26.5 ± 3.5	29.3 ± 3.9
3,200	19.8 ± 1.1	21.0 ± 1.2	23.4 ± 1.5	25.6 ± 1.7	28.8 ± 1.8
2,560	19.9 ± 1.0	20.6 ± 1.1	22.3 ± 1.2	24.8 ± 1.6	28.6 ± 2.3
2,048	19.7 ± 1.1	20.9 ± 1.4	23.1 ± 1.9	26.1 ± 2.2	29.2 ± 2.7

Note: Animal weights on the 0th day are the weights after fasting, and the number of animals corresponds to the number of surviving animals of each group shown in Table 2.

Table 2.

LD_{50} (Lethal Dosage, 50%) Results of Single Oral Administration of Metformin / Pioglitazone Compound (500: 7.5) to Mice

Dosage (mg/kg)	Number of Animals	Number of Mortality (♂)	Number of Mortality (♀)	Total No. of Mortality	Mortality Rate (%)	LD_{50} (Lethal Dosage, 50%) (mg/kg) (95% confidence limits set)
5,000	10	5	5	10	100	
4,000	10	3	5	8	80	3,137.3
3,200	10	3	3	6	60	(2,834.9 - 3,472.1)
2,560	10	1	1	2	20	
2,048	10	0	0	0	0	

Acute Toxicity Experiment of Metformin / Pioglitazone Compound (500: 15) Oral Administration:

Metformin / Pioglitazone compound (500: 15) was orally administered. 10 - 30 minutes after administration, reduction in animal activities was observed with some of the animals closing their eyes (1/10 to 4/10); one hour after administration, 3 animals demonstrated diarrhoea and 1 animal demonstrated asthenia of hind legs and a titubating gait; and two hours after administration, 9 animals demonstrated diarrhoea and 1 animal was in extremis. The time of occurrence of toxic reaction, the number of animals involved, and the degree of severity thereof appeared to be positively correlated with the dosage administered. Animal mortality first occurred 4 hours after administration and all animal mortalities occurred within 18 hours after administration. Animal anatomy indicated that some (4) of the animals had mild pneumonic hemorrhage, and no pathological lesions of organs were detected. 18 hours after administration, all surviving animals basically resumed normal activities. None of the surviving animals suffered mortality within the observation period of 14 days, and growth of animal weights was not distinctly affected. On the 14th day, some of the surviving animals were taken for anatomy and no pathological lesions were detected. LD_{50} (Lethal Dosage, 50%) of the oral administration to mice was 3,348.8 mg/kg. (Results are as shown in Table 3 and Table 4).

Table 3.

Effect of Single Oral Administration of Metformin / Pioglitazone Compound (500: 15) on Weight of Surviving Mice (g)

Dosage (mg/kg)	0th day	1st day	3rd day	7th day	14th day
5,000	19.7 ± 0.9	-	-	-	-
4,000	19.6 ± 1.1	20.3 ± 1.0	22.3 ± 1.0	26.0 ± 1.3	29.0 ± 2.6
3,200	19.5 ± 1.0	21.1 ± 1.3	23.4 ± 1.1	26.8 ± 1.9	29.7 ± 2.5
2,560	19.7 ± 1.0	21.2 ± 1.2	22.8 ± 1.7	26.2 ± 1.8	29.8 ± 2.0
2,048	19.7 ± 1.0	21.1 ± 1.1	23.4 ± 1.3	26.7 ± 1.6	29.8 ± 2.3

Note: Animal weights on the 0th day are the weights after fasting, and the number of animals corresponds to the number of surviving animals of each group shown in Table 4.

Table 4.
LD_{50} (Lethal Dosage, 50%) Results of Single Oral Administration of Metformin / Pioglitazone Compound (500: 15) to Mice

Dosage (mg/kg)	Number of Animals	Number of Mortality (♂)	Number of Mortality (♀)	Total No. of Mortality	Mortality Rate (%)	LD_{50} (Lethal Dosage, 50%) (mg/kg) (95% confidence limits set)
5,000	10	5	5	10	100	
4,000	10	3	4	7	70	3,348.8
3,200	10	3	2	5	50	(3,029.6 - 3,701.7)
2,560	10	0	1	1	10	
2,048	10	0	0	0	0	

Acute Toxicity Experiment of Metformin / Pioglitazone Compound (500: 30) Oral Administration:

Metformin / Pioglitazone compound (500: 30) was orally administered. 10 - 30 minutes after administration, reduction in animal activities was observed with some of the animals closing their eyes (1/10 to 4/10); one hour after administration, 4 animals demonstrated diarrhoea; and two hours after administration, 1 animal demonstrated a titubating gait and 7 animals demonstrated diarrhoea. The time of occurrence of toxic reaction, the number of animals involved, and the degree of severity thereof appeared to be positively correlated with the dosage administered. Animal mortality first occurred 5 hours after administration and all animal mortalities occurred within 18 hours after administration. Animal anatomy indicated that some (10) of the animals had mild pneumonic hemorrhage, and no pathological lesions of organs were detected. 18 hours after administration, all surviving animals basically resumed normal activities. None of the surviving animals suffered mortality within the observation period of 14 days, and growth of animal weights was not distinctly affected. On the 14th day, some of the surviving animals were taken for anatomy and no pathological lesions were detected. LD_{50} (Lethal Dosage, 50%) of the oral administration to mice was 3,726.7 mg/kg. (Results are as shown in Table 5 and Table 6).

Table 5.
Effect of Single Oral Administration of Metformin / Pioglitazone Compound (500: 30) on Weight of Surviving Mice (g)

Dosage (mg/kg)	0th day	1st day	3rd day	7th day	14th day
5,000	20.3 ± 1.0	-	-	-	-
4,000	20.2 ± 1.1	21.3 ± 1.2	22.8 ± 1.2	25.2 ± 1.3	28.8 ± 1.8
3,200	20.4 ± 0.9	21.9 ± 1.6	23.5 ± 2.0	26.2 ± 2.6	28.5 ± 2.4
2,560	20.3 ± 0.9	22.0 ± 0.5	24.1 ± 1.7	26.6 ± 2.3	29.7 ± 2.6
2,048	20.2 ± 1.1	21.9 ± 0.9	23.8 ± 1.0	26.7 ± 1.4	29.6 ± 2.5

Note: Animal weights on the 0th day are the weights after fasting, and the number of animals corresponds to the number of surviving animals of each group shown in Table 6.

Table 6.
LD_{50} (Lethal Dosage, 50%) Results of Single Oral Administration of Metformin / Pioglitazone Compound (500: 30) to Mice

Dosage (mg/kg)	Number of Animals	Number of Mortality (♂)	Number of Mortality (♀)	Total No. of Mortality	Mortality Rate (%)	LD_{50} (Lethal Dosage, 50%) (mg/kg) (95% confidence limits set)
5,000	10	5	5	10	100	
4,000	10	3	1	4	40	3,726.7
3,200	10	2	1	3	30	(3,338.6 - 4,159.8)
2,560	10	0	1	1	10	
2,048	10	0	0	0	0	

4. Conclusion

Single oral administration of Metformin / Pioglitazone compound of three different proportions to mice demonstrated a basically consistent toxic reaction. Some of the animals demonstrated reduction in activities and closing of eyes 10 - 30 minutes after administration, and some demonstrated diarrhoea and a titubating gait one hour after administration. The number of animals involved and the degree of severity of toxic reaction were positively correlated with the dosage administered. Animal mortality first occurred 4 - 5 hours after administration and all animal mortalities occurred within 18 hours after administration. Animal anatomy indicated that some of the animals had mild pneumonic hemorrhage, and no pathological lesions of organs were detected. 18 hours after administration, all surviving animals basically resumed normal activities. None of the surviving animals suffered mortality within the observation period of 14 days, and growth of animal weights was not distinctly affected. On the 14th day, some of the surviving animals were taken for anatomy and no pathological lesions were detected. LD_{50} (Lethal Dosage, 50%) of the oral administration of Metformin / Pioglitazone compound of different proportions (500: 7.5, 500: 15, and 500: 30) to mice were 3,137.3 mg/kg, 3,348.8 mg/kg, and 3,726.7 mg/kg.

II. Pharmacodynamic Study on The Anti-hyperglycemic Pharmaceutical - Metformin / Pioglitazone Compound

The anti-hyperglycemic pharmaceutical compound is a compound preparation of a biguanide anti-hyperglycemic agent Metformin and a thiazolidinone anti-hyperglycemic agent Pioglitazone, and is hereinafter referred to as "the anti-hyperglycemic pharmaceutical compound (500: 30, wt/wt). Due to working mechanism differences, the anti-hyperglycemic pharmaceutical compound is combined such that better anti-hyperglycemic effect is achieved. This study is intended to illustrate the similarity and the difference between the compound preparation and the single prescription preparations in absorption through experiment on absorption by rats.

1. Materials

(1) Pharmaceuticals

Metformin: Dissolved in water to a concentration of 15 mg/ml before use. Administration dosage: 1 ml/100 g body weight, equivalent to 150 mg/kg.

Pioglitazone: Suspended in 1% sodium carboxy methyl cellulose (SCMC) to a concentration of 0.9 mg/ml before use. Administration dosage: 1 ml/100 g body weight, equivalent to 9 mg/kg.

The anti-hyperglycemic pharmaceutical compound: Suspended in 1% sodium carboxy methyl cellulose (SCMC) to obtain 15 mg Metformin and 0.9 mg Pioglitazone per ml. Administration dosage: 1 ml/100 g body weight, equivalent to 150 mg/kg Metformin and 9 mg/kg Pioglitazone.

(2) Reagents

Methanol: Guaranteed reagent (GR); produced by Concord Technology (Tianjin) Co., Ltd., Batch No. 031204. Potassium dihydrogen phosphate: Analytical reagent (AR); produced by Beijing Hongxing Chemical Plant, Batch No. 851011-1. Biotin (B7): produced by Tianjin 2nd Chemical Reagent Plant. Acetonitrile: Analytical reagent (AR); produced by Concord Technology (Tianjin) Co., Ltd., Batch No. 031015. Sodium Acetate: produced by Tianjin Quartz Clock Plant Bazhou Chemical Branch, Batch No. 980303.

(3) Instruments

NL-200TPA Analytical Balance: Shimazu Company, Japan.

TGL-16C High Speed Table Centrifuge: Shanghai Anting Scientific Instrument Factory.

HPLC (High performance liquid chromatography): WATERS 515 Pump; 717 Automatic Liquid Injector; RAIMIN Ultraviolet Deector; ANASTAR Chromatorgraphy Data Workstation.

(4) Laboratory Animals

Healthy rats, female, weight approximately 210 g. Laboratory animal facilities certification - Laboratory Animal Facilities Approval No. 013 issued by Tianjin Laboratory Animals Administration Committee, meeting requirements for Class 1. Animals were fed as usual for three days before experiment.

2. Method

(1) Sample Collection and Treatment

Before experiment, 12 female healthy rats were made to fast for 16 hours and divided into 3 groups, i.e. 9 mg/kg Pioglitazone group, 150 mg/kg Metformin group, and anti-hyperglycemic pharmaceutical compound group. At 8:00 o'clock, the above pharmaceuticals were orally administered to the groups respectively. 0.5 ml orbital blood samples were collected at 0.33, 0.66, 1.0, 1.5, 2.0, 4.0, 6.0, 12.0, 24.0 and 36.0 hours after administration, and centrifugal separations of serum were carried out.

50 μl of serums were quantitatively extracted from animals of the Metformin group and the anti-hyperglycemic pharmaceutical compound group, and equal volume of 10% perchloric acid was added. Thorough shaking was performed to ...

... precipitate proteins, and this was followed by centrifugation, addition of 20 µl supernatant liquid and high performance liquid chromatographic analysis. Blank serum tube operation was performed to prepare standard serum samples with concentrations of 0 µg/ml, 0.5 µg/ml, 1 µg/ml, 2 µg/ml, 5 µg/ml, 10 µg/ml, and 20 µg/ml for standard curve, and the method of processing was as earlier stated.

150 µl of serums were quantitatively extracted from animals of the Pioglitazone group and the anti-hyperglycemic pharmaceutical compound group, and 1 ml of dichloromethane (DCM) was added. Thorough shaking and centrifugation were performed, and 800 µl of substance in the bottom organic phase was placed in another centrifuge tube and subjected to air drying. 75 µl was re-dissolved in mobile phase, and this was followed by centrifugation, addition of 20 µl sample and high performance liquid chromatographic analysis. Blank serum tube operation was performed to prepare standard serum samples with concentrations of 0 µg/ml, 0.1 µg/ml, 0.5 µg/ml, 1 µg/ml, 5 µg/ml, and 10 µg/ml for standard curve, and the method of processing was as earlier stated.

(2) Chromatography conditions

Metformin:
Stationary Phase: C_{18} ODS Column, 4.6 x 250 mm, 10 µ, Column No. 22I25117
Mobile Phase: Methanol: 0.005M Potassium Dihydrogen Phosphate (pH 2.5) = 10: 90
Column Temperature: 40° C
UV Detection: 233 nm

Pioglitazone:
Stationary Phase: C_{18} ODS Column, 4.6 x 100 mm, 5 µ, Column No. 22K10040
Mobile Phase: Acetonitrile: 0.1M Sodium Acetate (pH 4.5) = 39: 61
Column Temperature: 30° C
UV Detection: 269 nm

(3) Experiment Results

Metformin

Linear equation of serum standard curve (0 ~ 20 µg/ml) - C = 0.0000353A + 0.10355 (r = 0.9992). Recovery rate was 95.04%. Blood drug concentrations of rats at different times after oral administration of Metformin and the anti-hyperglycemic pharmaceutical compound are shown in Table 7. Concentration - time curve is shown in Figure 1. Average peak times were 1.1 hours and 1.0 hour respectively. Peak concentrations were 19.6 µg/ml and 20.4 µg/ml respectively. Areas under the concentration - time curve were 239.8 µg•h/ml and 249.9 µg•h/ml respectively. Relative bioavailability of the anti-hyperglycemic pharmaceutical compound with respect to single preparation was 101.7%.

Table 7. Blood Drug Concentrations of Rats at Different Times After Oral Administration of Metformin and the Anti-hyperglycemic Pharmaceutical Compound

Group	Blood Drug Concentration (μg/ml) with respect to Time										
	0.33 h	0.67 h	1 h	1.5 h	2 h	4 h	6 h	8 h	12 h	24 h	36 h
Metformin (Single Preparation)											
1	13.5	16.3	19.3	19.3	16.1	8.3	8.2	6.7	6.0	3.1	3.5
2	12.2	15.1	24.6	20.0	16.9	9.3	5.5	5.4	10.4	13.1	2.3
3	14.5	13.7	14.5	18.4	13.7	10.8	5.8	5.2	5.6	4.6	2.8
4	13.6	15.3	16.3	14.0	14.9	7.2	5.1	4.9	4.7	5.4	1.9
Mean	13.4	15.1	18.7	17.9	15.4	8.9	6.2	5.5	6.7	6.6	2.6
Anti-hyperglycemic Pharmaceutical Compound											
1	12.6	19.6	27.2	21.6	14.9	14.7	8.5	7.3	6.8	6.6	2.5
2	12.5	15.6	16.3	15.1	15.9	10.2	6.9	6.5	6.5	5.7	2.9
3	11.6	18.7	19.1	16.1	16.2	10.7	7.3	6.3	5.3	4.3	2.6
4	15.9	17.9	19.1	15.4	18.8	10.3	8.0	6.8	6.7	6.8	3.4
Mean	13.1	18.0	20.4	17.1	16.4	11.5	7.7	6.7	6.3	5.8	2.8

Pioglitazone

Linear equation of serum standard curve (0 ~ 10 μg/ml) - C = 0.0000310A + 0.1488 (r = 0.9988). Recovery rate was 71.7%. Blood drug concentrations of rats at different times after oral administration of Pioglitazone and the anti-hyperglycemic pharmaceutical compound are shown in Table 8. Concentration - time curve is shown in Figure 2. Times of average peak were 4.0 hours and 3.9 hours respectively. Peak concentrations were 6.8 μg/ml and 5.3 μg/ml respectively. Areas under the concentration - time curve were 80.4 μg•h/ml and 85.0 μg•h/ml respectively. Relative bioavailability of the anti-hyperglycemic pharmaceutical compound with respect to single preparation was 105.7%.

Table 8. Blood Drug Concentrations of Rats at Different Times After Oral Administration of Pioglitazone and the Anti-hyperglycemic Pharmaceutical Compound

Group	Blood Drug Concentration (μg/ml) with respect to Time										
	0.33 h	0.67 h	1 h	1.5 h	2 h	4 h	6 h	8 h	12 h	24 h	36 h
Pioglitazone (Single Preparation)											
1	0.68	2.95	3.49	5.32	3.71	4.72	4.36	4.13	2.48	0.36	nd
2	5.84	10.81	8.09	10.73	7.60	7.32	5.90	5.12	3.25	0.66	0.34
3	3.51	2.84	3.62	3.96	4.85	4.47	4.93	4.07	3.10	0.69	0.42
4	0.81	2.36	3.00	3.23	3.74	4.54	6.06	6.14	3.57	0.39	nd
Mean	2.71	4.74	4.55	5.81	4.97	5.26	5.31	4.87	3.10	0.52	0.38
Anti-hyperglycemic Pharmaceutical Compound											
1	1.05	2.29	2.80	2.87	2.85	3.26	2.38	1.86	1.12	2.58	0.40
2	0.42	0.46	3.16	3.81	3.19	5.49	5.95	5.09	3.41	1.73	2.47
3	0.44	3.37	4.21	5.41	5.48	5.95	5.09	3.40	1.68	2.44	0.69
4	3.33	4.21	5.41	5.93	4.57	3.64	5.28	3.22	1.85	0.80	1.31
Mean	1.31	2.58	3.90	4.51	4.02	4.59	4.67	3.39	2.01	1.89	1.22

3. Conclusion

Average peak times after oral administration of Metformin and the anti-hyperglycemic pharmaceutical compound were 1.1 hours and 1.0 hour respectively. Peak concentrations were 19.6 μg/ml and 20.4 μg/ml respectively. Areas under the concentration - time curve were 239.8 μg•h/ml and 249.9 μg•h/ml respectively. I respectively. Areas under the concentration - time curve were 80.4 μg•h/ml and 85.0 μg•h/ml respectively. Relative bioavailability of the anti-hyperglycemic pharmaceutical compound with respect to single preparation was 101.7%. Average peak times after oral administration of Pioglitazone and the anti-hyperglycemic pharmaceutical compound were 4.0 hours and 3.9 hours respectively. Peak concentrations were 6.8 μg/ml and 5.3 μg/ml respectively. Areas under the concentration - time curve were 80.4 μg•h/ml and 85.0 μg•h/ml respectively. Relative bioavailability of the anti-hyperglycemic pharmaceutical compound with respect to single preparation was 105.7%. In vivo absorption of the two anti-hyperglycemic pharmaceuticals contained in the anti-hyperglycemic pharmaceutical compound by rats was basically not interfered and not distinctly different from those of the single preparations.

Brief Description of the Drawings

Figure 1 is a concentration - time curve showing blood drug concentrations of rats at different times after oral administration of Metformin and the anti-hyperglycemic pharmaceutical compound.

Figure 2 is a concentration - time curve showing blood drug concentrations of rats at different times after oral administration of Pioglitazone and the anti-hyperglycemic pharmaceutical compound.

III. Pharmacodynamic Experiment of Metformin / Pioglitazone Compound

1. Experiment Materials

1.1 Animals for Experiment

Wistar rats - weight ranging from 140 ~ 160 g. Laboratory animal certification: W-J Jin Shi Dong Zhi R Zhun No. 001.

1.2 Laboratory Environment and Conditions

Laboratory animal facilities: Class 2; Certification Number: Jin Shi Dong Sheshi Zhun No. 012 (Tianjin Laboratory Animal Facilities Approval No. 012); Room temperature 22±4 °C, relative humidity 60±20%. Centralized air-conditioning with automatic ventilation. Illumination was provided for 12 hours. Food and piped water were self fed. Water was replaced once a day.

1.3 Pharmaceuticals for Experiment

Metformin Hydrochloride; Pioglitazone Hydrochloride. The two pharmaceuticals were ground to become powder and thoroughly mixed. 1% carboxy methyl cellulose (CMC) was used to prepare medicinal suspension.

1.4 Reagents and Instruments

Streptozotocin (STZ, Sigma S-0130), imported packaging; supplied by Beijing Xin Jing Ke Biotechnology Co., Ltd. Specification: 1 g/bottle. Purity: 98%.

Kyoto Daiichi Kagaku blood glucose meter (Super Glucocard II) and test strips, produced in Japan and supplied by Beijing Mai Bang Biotechnology Co., Ltd.

Insulin reagent kit supplied by Tianjin Shu Pu Biological Engineering Co., Ltd. Batch No. 06043-A.

Sunrise enzyme sign analyzer produced by TECAN.

2. Experiment Method and Results

300 male Wistar rats with weight ranging from 140 g ~ 160 g were made to fast for 16 hours. Abdominal injection of 30 mg/kg Streptozotocin (STZ) (dissolved in 0.1 mol/L pH 4.4 citrate buffer in 4 ° C ice bath, used immediately after preparation) was given once a day for continuously 3 days. Two weeks after administration, high-fat high-sucrose feed (base feed 55%, pig lard 25%, sucrose 20%) was given. After 6 weeks of feeding, fibrinogen (FBG) was measured (rats were made to fast for 12 hours before measurement). 64 rats with fibrinogen (FBG) ≥ 12.0 mmol/L were selected and randomly divided into 8 groups with each group having 8 rats. The groups were the model control group, 300: 1.5 pharmaceutical compound group, 300: 3 pharmaceutical compound group, 300: 4.5 pharmaceutical compound group, 300: 6 pharmaceutical compound group, 300: 6.75 pharmaceutical compound group, 300: 9 pharmaceutical compound group, and 300: 27 pharmaceutical compound group respectively. Another 8 normal male Wistar rats were taken as the biological control group (taken from the same batch of rats, fibrinogen (FBG) ≤ 5.0 mmol/L). The biological control group and the model control group were fed with 1% carboxy methyl cellulose (CMC), and the seven pharmaceutical compound groups were fed with 300 mg: 1.5 mg/kg, 300 mg: 3 mg/kg, 300 mg: 4.5 mg/kg, 300 mg: 6 mg/kg, 300 mg: 6.75 mg/kg, 300 mg: 9 mg/kg, and 300 mg: 27 mg/kg of the pharmaceutical compound (Metformin Hydrochlorid: Pioglitazone Hydrochloride). Suspensions of different concentrations were prepared with the tested pharmaceuticals and 1% carboxy methyl cellulose (CMC). Immunoglobulin administration was given every morning, and administration volume was 1 ml/100g. The biological control group and the model control group were administered with equal volume of 1% carboxy methyl cellulose (CMC). Administration was given for 21 days. At 9:00 am on the 22nd day, 1 drip of blood sample was taken from plexus venocus in eyeground using capillary glass tube (animals were made to fast for 12 hours before blood sampling), and fibrinogen (FBG) was measured with blood glucose meter. Another 1 ml of blood sample was taken before obtaining serum by centrifugation. Fasting serum insulin (FINS) was measured using ELASE method according to the instructions on the reagent kit. The following computations were made: absolute glucose reduction value (absolute glucose reduction value = FBG on the 21st day - FBG before administration), glucose reduction rate (glucose reduction rate = [(FBG on the 21st day - FBG before administration) ÷ FBG before administration x 100%]), and insulin resistance index IR (insulin resistance index IR = FBG x FINS ÷ 22.5). The various data are expressed in the form of average value ± standard deviation. The various groups and the model control group were compared based on inter-group t tests. Results: ...

... The pharmaceutical compounds of various proportions had to different extents reduced fibrinogen (FBG) of the hyperglycemic rats. Comparing the pharmaceutical compound groups with dosage above 300 mg: 3 mg/kg with the model control group, their absolute glucose reduction values and glucose reduction rates are distinctly different and demonstrated dosage correlation. The pharmaceutical compounds of various proportions are capable of distinctly reducing the fasting serum insulin (FINS) and insulin resistance (IR) levels of the hyperglycemic rats. See Table 9 and Table 10 for details.

Table 9. Effect of Metformin Hydrochloride - Pioglitazone Hydrochloride Compound on Fasting Blood Glucose of Rats

Group	Number of Animals	FBG Before Administration (mmol/L)	FBG on 21st Day of Administration (mmol/L)	FBG Difference (21st – 1st Day) (mmol/L)	Glucose Reduction Rate on 21st Day (%)
Blank Control	8	3.7 ± 0.5 ***	3.8 ± 0.5 ***	0.1 ± 0.4	2.7 ± 11.7
Model Control	8	20.5 ± 3.9	20.0 ± 3.5	-0.5 ± 1.3	-2.1 ± 6.0
Compound 300: 1.5	8	20.3 ± 5.2	17.9 ± 3.9	-2.4 ± 2.5	-10.7 ± 10.1
Compound 300: 3	8	20.5 ± 4.9	17.6 ± 3.2	-2.9 ± 2.4 *	-12.8 ± 8.8 *
Compound 300: 4.5	8	20.4 ± 4.3	17.7 ± 4.3	-2.7 ± 2.6	-13.1 ± 11.5 *
Compound 300: 6	8	20.5 ± 4.2	17.3 ± 6.0	-3.2 ± 2.8 *	-17.3 ± 16.4 *
Compound 300: 6.75	8	20.3 ± 4.0	16.4 ± 3.0	-3.9 ± 2.2 **	-18.7 ± 8.5 ***
Compound 300: 9	8	20.5 ± 5.5	15.9 ± 4.3	-4.6 ± 2.3 ***	-22.5 ± 9.4 ***
Compound 300: 27	8	20.3 ± 3.6	16.2 ± 3.4	-4.1 ± 2.5 **	-20.0 ± 12.2 **

Note: In comparison with the model control group, * $p < 0.05$, ** < 0.01, *** $p < 0.001$

Table 10.
Effect of Metformin Hydrochloride - Pioglitazone Hydrochloride Compound on Fasting Serum Insulin (FINS) and Insulin Resistance (IR) of Rats

Group	Number of Animals	FBG on 21st Day of Administration (mmol/L)	FINS on 21st Day of Administration (mmol/L)	Insulin Resistance Index on 21st Day of Administration (Logarithmic)
Blank Control	8	3.8 ± 0.5 ***	3.86 ± 0.82 ***	-0.20 ± 0.12 ***
Model Control	8	20.0 ± 3.5	32.35 ± 15.93	1.40 ± 0.29
Compound 300: 1.5	8	17.9 ± 3.9	8.80 ± 4.63 **	0.79 ± 0.25 ***
Compound 300: 3	8	17.6 ± 3.2	6.53 ± 4.00 **	0.65 ± 0.22 ***
Compound 300: 4.5	8	17.7 ± 4.3	12.68 ± 9.63 **	0.87 ± 0.31 **
Compound 300: 6	8	17.3 ± 6.0	8.27 ± 4.56 **	0.70 ± 0.25 ***
Compound 300: 6.75	8	16.4 ± 3.0	7.28 ± 4.04 **	0.66 ± 0.25 ***
Compound 300: 9	8	15.9 ± 4.3	9.18 ± 5.00 **	0.72 ± 0.37 ***
Compound 300: 27	8	16.2 ± 3.4	9.64 ± 4.33 **	0.79 ± 0.25 ***

Note: In comparison with the model control group, * $p < 0.05$, ** < 0.01, *** $p < 0.001$

3. Conclusion

Metformin Hydrochloride - Pioglitazone Hydrochloride compounds (300 mg: 1.5 mg/kg, 300 mg: 3 mg/kg, 300 mg: 4.5 mg/kg, 300 mg: 6 mg/kg, 300 mg: 6.75 mg/kg, 300 mg: 9 mg/kg, and 300 mg: 27 mg/kg) were orally administered continuously for 21 days, and the pharmaceutical compounds of various proportions had to different extents reduced fibrinogen (FBG), fasting serum insulin (FINS) and insulin resistance (IR) levels of the hyperglycemic rats. The reductions in fasting serum insulin (FINS) and insulin resistance (IR) levels were especially distinct.

Detailed Description of the Invention

The present invention is further described in connection with the following embodiments which should not be misconstrued as limiting the scope of the invention. In order to better illustrate the implementation of the present invention, the following exemplary preparations are provided. The preparations may take the form of any of the pharmaceutical compounds of the present invention. The Metformin Hydrochloride - Pioglitazone Hydrochloride compounds of the following proportions are taken as exemplary preparations: (1) 500: 15; (2) 750: 15; and (3) 1,000: 15.

Preparation 1

Two-layer (top and bottom) tables:

Top Layer Composition	Content per Tablet	Percentage Concentration (wt) (%)
Pioglitazone Hydrochloride	15 mg	12.1
Microcrystalline Cellulose	55 mg	44.4
Lactose	45 mg	36.3
Polyvinylpyrrolidone (PVP)	3 mg	2.4
Hydroxymethyl Starch Sodium	4.5 mg	3.6
Magnesium Stearate	0.5 mg	0.4
Talcum Powder	1 mg	0.8

Bottom Layer Composition	Content per Tablet	Content per Tablet	Content per Tablet
Metformin Hydrochloride	500 mg	750 mg	1,000 mg
Hydroxypropyl Methyl Cellulose (HPMC)	190 g	205 mg	210 mg
Polyvinylpyrrolidone (PVP)	14.0 mg	19.1 mg	24.2 mg
Magnesium Stearate	7.1 mg	9.7 mg	12.3 mg

Preparation Method:

Method for preparation of Pioglitazone Hydrochloride particles: Sieve active ingredients, lactose, and microcrystalline cellulose and mix thoroughly. Mix the said powder with polyvinylpyrrolidone (PVP) solution. Sieve and dry obtained moist particles at 50 ~ 60 °C. Sieve hydroxymethyl starch sodium, magnesium stearate and talcum powder and add them to the said particles.

Method for preparation of Metformin Hydrochloride particles: Sieve active ingredients and hydroxypropyl methyl cellulose (HPMC), and mix thoroughly. Mix the said powder with polyvinylpyrrolidone (PVP) ethanol solution. Sieve and dry obtained moist particles at 50 ~ 60 °C. Add magnesium stearate to the said particles.

Place the said particles on double-layer tablet pressing machine to produce tablets.

Preparation 2

Two-layer (inner and outer) tables:

Inner Layer Composition	Content per Tablet	Content per Tablet	Content per Tablet
Metformin Hydrochloride	500 mg	750 mg	1,000 mg
Hydroxypropyl Methyl Cellulose (HPMC)	190 g	205 mg	210 mg
Polyvinylpyrrolidone (PVP)	14.0 mg	19.1 mg	24.2 mg
Magnesium Stearate	7.1 mg	9.7 mg	12.3 mg

Outer Layer Composition	Content per Tablet
Pioglitazone Hydrochloride	15 mg
Hydroxypropyl Methyl Cellulose (HPMC)	43.6 mg
Polyethylene Glycol (PEG) 400	4.5 mg

Preparation Method:

Method for preparation of slow-release Metformin Hydrochloride tablet cores: Sieve active ingredients and hydroxypropyl methyl cellulose (HPMC), and mix thoroughly. Mix the said powder with polyvinylpyrrolidone (PVP) ethanol solution. Sieve and dry obtained moist particles at 50 ~ 60 °C. Add magnesium stearate to the said particles. Press to produce tablet cores.

Pioglitazone Hydrochloride coating fluid: Mix Pioglitazone Hydrochloride and hydroxypropyl methyl cellulose (HPMC) thoroughly. Dissolve suitable amount of polyethylene glycol (PEG) 400 in water and slowly add in the said mixture while stirring to obtain a liquid suspension. The coating fluid contains approximately 9% solid.

Sieve the liquid suspension through 80 mesh screen filter and coat tablet core under suitable conditions. Control weight gain so that each tablet contains approximately 15 mg of Pioglitazone Hydrochloride.

Though the present invention has been described in detail in connection with the implementation embodiments thereof, alteration and modification can be made by persons skilled in the art without departing from the spirit and scope of the invention.